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Biochemical and mineral composition and bacteriological study in some selected fresh water fishes in Meghna river of Bangladesh

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Abstract
Fish play an important role in the Bangladeshi diet, constituting the main and often irreplaceable animal-source food in poor rural households. Study on the biochemical, mineral-compositions and bacteriology of three different freshly-caught fishes were conducted using standard procedures. The fishes are *Channa striatus* (shol), *Channa punctatus* (taki) and *Mystus tengra* (tengra). The results revealed the mean moisture contents were 77.03%, 78.05% and 74.27%; protein contents were 19.52%, 16.89% and 16.96%; fat contents were1.93%, 2.50% and 6.04%; ash contents were 1.44%, 1.36 and 2.67% respectively for *C. striatus*, *C. punctatus* and *M. tengra*. Chemical-parameters and Total bacterial count were in acceptable limits. Minerals included calcium (11.2-22.02 mg/g) and magnesium (9.4-10.4 mg/g) while iron, zinc, copper and manganese were present in trace amounts. The data has proved with strong evidence that the fishes undertaken for the present study are rich in most of the nutrients essential for proper health maintenance of humans.

Keywords: Freshwater fishes, bio-chemical composition, minerals, bacteriological-study

1. Introduction
Fish is a non-tetrapod chordate, i.e., an animal with a backbone that has gills throughout its life and has limbs in the shape of fins [1]. It is one of the best sources of proteins, vitamins and minerals and are essential nutrients required for supplementing both infant and adult diets [2].
It is well known fact that Rice and Fish dominate the diet of Bangladeshi peoples. In terms of weight of food consumed, fish ranks third after rice and vegetables [3, 4]. In Asia, Bangladesh is ranked third largest aquaculture producing country after China and India [5].According to DoF, Fisheries provide 63% of animal protein along with other nutrients like vitamins, minerals etc., and contributed 3.74% GDP and 2.46% foreign exchange earnings in 2011-2012 fiscal year [5].
Among the freshwater fish species, *Channa striatus* (Shol) and *Channa punctatus* (Taki) are delicious, nutritious and popular to the consumers as well as bear high market price. Another popular lean catfish *Mystus tengra* (Tengra) is selected for the present study which is one of the sole species of family Bagridae and a common catfish of the commercial catches of Bangladesh. These fishes have unique test and high demand from all corners of the country as these are economical in price and full of nutrients especially animal protein and fats.
The Bio-chemical composition is an important aspect of fish quality and its influences both the keeping quality and technological characteristics of fish. The knowledge of fish composition is essential for its maximum utilization. The nutritional composition of fish varies greatly from one species and individual to another, depending on age, feed intake, sex and sexual changes connected with spawning, the environment and season [6]. The chemical composition could influence the postharvest processing and storage and could assist in determining the suitability of the different fishes to specific processing and storage techniques.
Now-a-days, large group of consumers have become more health conscious and interested in convenience food. The changing pattern of life style and increasing number of households in the rural area have an impact on the market demands since consumers now a days insist that the product should be acceptable in respect both quality and safety.
In Bangladesh, very little work has been done on the presence of macro and micro elements in freshwater fish, despite such data are important to assess the quality and safety of fish and fishery products for domestic consumption as well as for export. For above reason, the present investigation was carried out to determine the biochemical and mineral composition of three freshwater fishes and study their total bacterial load.

2. Materials and methods
2.1. Collection of experimental fishes: Fresh mature fish samples were collected from fishermen of Meghna River in the early hours of the day.

2.2. Handling of experimental fish in laboratory: Being air breathing fish, *Channa striatus* (Shol) and *Channa punctatus* (Taki) fish were transported to the research laboratory in dram full with water. In case of *Mystus tengra* (Tengra) fish, they were carried in clean, good quality polythene bag with ice in order to keep the fish fresh.

2.3. Place/location of the experiment: Biochemical and Microbial analysis was carried out at the 'Fish Technology Section' and 'Food Microbiology section' of the Institute of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka and from center for Advanced Research in Sciences (CARS) for Minerals.

2.4. Preparation of fish: The fishes were carefully washed with cooled tap water. Fins, scales, gills and viscera were removed and again washed with tap water to remove blood, slime and unnecessary flesh. Because of hard and large bones of the head, the bones and head of *C. striatus* and *C. punctatus* are included as the waste.

2.5. Sampling procedure: 6 or 7 slice of 3 experimental fish species was taken randomly which represented the parts from whole body of the fish. Then the slices were chopped with skin and bone and finally ground with an electric blender to make a homogenous sample before being sampled for analysis. Triplicate experiments were conducted for this analysis.
Analytical methods were applied for the determination of biochemical composition of the raw fishes on experimental basis.

2.6. Proximate composition analysis: Proximate compositions of fish were determined by conventional method of AOAC (Association of Official Analytical Chemicals) on weight basis [7].

2.6.1. Estimation of moisture: About 5 gram of previously prepared fairly minced samples were taken into each known weight basin and weighed in a digital balance (Toledo, Switzerland). The samples were allowed to dry into the oven (Memmet 854 Schwabach) at 105^0C for 24 hours in order to remove the moisture until constant weight. After that, the basins are taken out of the oven, cooled in a desiccators and were weighed in a digital balance.

Calculation

$$\% \text{ of Moisture} = \frac{\text{Weight Loss}}{\text{Original Weight of Sample Taken}} \times 100 \quad (1)$$

2.6.2. Estimation of protein: The protein content of the fish was determined by micro-kjeldahl method [8]. It involves conversion of organic nitrogen to ammonium sulphate by digestion with concentrated sulphuric acid in a microkjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and was determined titrametrically.

Calculation
The percentage of nitrogen in the sample was calculated by the following equation:

$$\frac{\%\text{ of } N_2(\text{titration reading - blank reading}) \times \text{strength of acid} \times 0.002 \times \frac{100}{5} \times 100}{\text{weight of sample taken}} \quad (2)$$

For most routine purposes the percent of protein in the sample is then calculated by multiplying the % of N_2 with the protein conversion factor of 6.25 for fish.
% of protein = % of total $N_2 \times 6.25$

2.6.3. Estimation of fat: About 5 g of the homogenous sample was taken into conical flasks and 10 ml of folch reagent (Chloroform: Methanol = 2:1) was added into the sample and homogenized properly and kept in air-tight condition for 24 hours. Fat contents of the fish muscle react with that solvent and remains in the solution. After 24 hours the solution of the flask was filtered in another weighed conical flask through a filter paper. Then these flasks were given in a hot water bath to dry up and removed the solvent. After that the flasks were kept into an oven for an hour to get the actual fat content. Then the flasks were weighed in an electronic balance to get the amount of fat content.

Calculation

$$\% \text{ of Fat} = \frac{\text{Weight of the residue}}{\text{Weight of sample taken}} \times 100 \quad (3)$$

2.6.4. Estimation of ash: About 4-5 g fish sample was weighed into a pre-weighed crucible. The crucible with the contents was heated first over a long flame till all the material was completely churned. Then it was transferred in the Muffle Furnace held at dark red at a rate of 600^0C for 5 hours until the residue become white. The crucible were cooled in desiccators and weighed. Finally the % of ash content was calculated.

Calculation

$$\% \text{ of ash} = \frac{\text{Weight of fish}}{\text{Weight of sample taken}} \times 100 \quad (4)$$

2.7. Estimation of Chemical Composition: To determine the quality of fishes, some parameters, *viz.* TVB-N value, pH, FFA, etc. were analyzed.

2.7.1. Estimation of TVB-N (Total Volatile Base Nitrogen): TVB-N has been used as an index for the determination of freshness of fish [9, 10]. Volatile nitrogenous bases increase in concentration during the spoilage of fish [11]. The TVB-N measurement can be used as a parameter for the determination of microbiological and enzymatic spoilage of fish product. TVB-N value was determined by using Conway modified micro-diffusion technique [12]. Samples that were in the different levels of acceptability from highly acceptable condition to unacceptable condition had been selected for TVB-N analysis. 25 ml of 10% Trichloro Acetic Acid (TCA) was added to 2 gm of ground fish sample and kept overnight and then filtrated with known volume. 2% boric acid, TCA, K_2CO_3 and the solutions made from the fish samples were taken into the Conway dishes. After the addition of Potassium Carbonate (K_2CO_3), each dish was covered by a piece of glass that was stacked with glue (Paraffin soft white) initially. Then it was kept for 24 hours. The samples and Potassium Carbonate (K_2CO_3) reacts to form NH_3 which was absorbed by the boric acid and then the solution of each Conway dish had been titrated by N/70 H_2SO_4 with the help of a micro-burette. Finally TVB-N was calculated.

Calculation

$$TVN = (\text{titration reading} - \text{blank reading}) \times \text{Strength of acid} \times 0.2 \times \frac{\text{Volume of extract}}{\text{Volume of extract taken}} \times \frac{100}{\text{Weight of sample taken}} \quad (5)$$

2.7.2. Estimation of pH: A 1 g sample of the fish flesh was homogenized in 10 ml of distilled water and the mixture was filtered. The pH of the filtrate was measured using a pH meter (Mettler Toledo 320-s, Shanghi, China) [13].

2.7.3. Free Fatty Acid (FFA) Estimation: Oil sample used throughout the work was prepared by extracting the fish by Folch reagent (chloroform and methanol in the ratio of 2:1 v/v).The fish was first cut into small pieces and then ground. The ground material was then mixed with folch reagent in a large wide mouthed stopper glass bottle for 24 hours at room temperature after 15 minutes stirring with glass rod. Extraction was facilitated by occasional shaking. The mixture was filtered through a Buchner funnel and the filtrate was evaporated in batches under heater or oven at 60°C. Seven gram of well-mixed oil was taken into 250 ml flask and 50 ml ethanol was added, previously neutralized by adding 2 ml phenolphthalein solution. Tritation was done by 0.25 N sodium hydroxide with vigorous shaking until permanent final faint pink color appeared and persisted for at least one minute. The value was reported as percentage (%) of free fatty acid expressed as oleic acid. Milli litre of 0.25 N NaOH required for titration corresponds to the percentage of free fatty acid.

2.8. Mineral determination: Samples for mineral analysis were prepared according to recommendations of Perkin Elmer's procedures of Atomic Absorption Spectrometer [14].

2.9. Estimation of total bacterial load: Enumeration of bacterial load was done using plate count agar by pour plate techniques. 10 g of the sample was mixed with 90 ml of previously sterile ringer solution. Appropriate dilutions of homogenate-fish samples was then transferred to petri dish and mixed with plate count agar and incubated at 37°C for 24 hours and the colonies were counted for total Plate count and the count was expressed as CFU/g [15].

Statistical analysis: Data were analyzed by using SPSS for windows-20 statistical programme. Significance was established at $p< 0.05$ [16].

3. Results & Discussion

3.1 Bio-chemical Composition: Bio-chemical composition of the fresh experimental fish, *Channa striatus* (shol), *Channa punctatus* (taki) and *Mystus tengra* (tengra) is given Table-1.

Table 1: Mean initial bio-chemical composition of the fresh experimental fish, *Channa striatus* (shol), *Channa punctatus* (taki) and *Mystus tengra* (tengra)

Parameters	*C. striatus*	*C. punctatus*	*M. tengra*
Moisture (%)	77.03±.12^{a}	78.65±.07^{b}	74.27±.07^{c}
Protein (%)	19.52±.07^{a}	16.89±.10^{b}	16.96±.07^{b}
Fat (%)	1.93±.07^{a}	2.50±.06^{b}	6.04±.06^{c}
Ash (%)	1.44±.11^{a}	1.36±.11^{a}	2.67±.08^{c}
TVB-N	4.41±.01^{a}	3.43±.02^{b}	4.27±.02^{c}
FFA (Free fatty acid)	0.6±.06^{a}	0.5±.1^{ba}	0.9±.21^{ca}
pH	6.9±.06^{a}	7.0±.06^{a}	7.0±.1^{a}

Values are shown as mean±standard deviation of triplicate measurements; a, b, c: Means significant differences between groups. Common superscript letter within a row (horizontal) are not significantly different ($p>0.05$) from each other using LSD test.

The major component of fish muscle was found to be moisture.The moisture contents of *C. striatus, C. punctatus* and *M. tengra* fish in fresh raw condition was found 77.03±.12, 78.65±.07 and 74.27±.07% respectively which resembles with the findings that moisture content of fresh water fish ranged from 70-80% [17].

The protein (%) of *C. striatus, C. punctatus* and *M. tengra* fish in fresh raw condition was 19.52±.07, 16.89±.10 and 16.96±.07% which have got more or less similarities with the findings of the results of *M. aculeatus,G. chapra and P. chola*[18, 19]. Salam reported that, the protein in *H. fossilis* was 18.25% which was similar with findings of *C. striatus,* but higher compared with the findings of *C. punctatus* and *M. tengra* and this might be to species variation [20]. All the fish species examined belonged to high-protein group of fish which resembles with the findings that protein content of fresh water fish ranged from 18-23% [21].

In fresh raw *C. striatus, C. punctatus* and *M. tengra* fish, fat content was 1.93±.07, 2.50±.06 and 6.04±.06% respectively which was more or less similar to the findings of Habashy in *C. carpio,* Nabi and Hossain in *P. gonionotus* and of Mazumder in *G. chapra,P. chola, A. coila* and *A.mola* [22, 18, 19].Salam estimated the highest fat content as 3.25% in *H. fossilis* which was less than that of *M. tengra*[20]. These differences might be due to the reason of species variation. According to Ackman, fish can be grouped into four categories according to their fat content: lean fish (< 2 %), low fat (2 to 4 %), medium fat (4 to 8%), and high fat (> 8%) [23]. Thus, *C. striatus* and *C. punctatus* can be grouped as low fat fish whereas *M. tengra* can be treated as medium fat fish.

The value of ash in *C. striatus, C. punctatus* and *M. tengra* fish was 1.44±.11, 1.36±.11and 2.67±.08% respectively which was nearer to the values obtained by Mazumderin *Aila coila* &*A. mola* andby Salamin *H. fossilis*[19, 20]. The present findings state that the ash contents of these three freshwater fishes might be a good source of minerals such as calcium, magnesium, iron, copper, zinc, manganese etc. Findings of this experimentin respect of fat and ash content of fresh raw fish have got similarities with the findings of Srivastava and Balachandran who has got percent of fat and ash ranged from 1.0-7.0 and 0.4-3.0 respectively [24, 25].From the table it is clear that fresh fish samples presented a higher moisture and low protein content [26].

TVB-N (total volatile base nitrogen) contents of fresh *C. striatus, C. punctatus* and *M. tengra* fish was 4.41±.01, 3.43±.02 and 4.27±.02 mgN/100g respectively. According to EEC the TVB-N value of raw fish was much lower than the acceptable upper limits of 25-35 mg/100 g for some fish species [27]. This is in agreement with the initial TVB-N values of these three fishes.

FFA (%) (Free fatty acid) value of fresh *C. striatus, C. punctatus* and *M. tengra* fish was 0.6±.06%, 0.5±.1% and 0.9±.21% respectively. The acceptable limit of FFA in fresh fish is about 0.5-1.5% [28]. The FFA (%) value in this experiment with three fish species showed the values within the range of acceptable limit.

pH value of fresh *C. striatus, C. punctatus* and *M. tengra* fish was 6.9±.06, 7.0±.06 and 7.0±.1 respectively. The pH in fresh condition fresh-water fish flesh is almost neutral [10]. This is in agreement with the initial pH values of these three fishes.

From the table it is clear that Moisture, fat and TVB-N content of *C. striatus, C. punctatus* and *M. tengra* fish are significantly different ($p<0.000$). But, in case of protein, no significant difference was shown between *C. punctatus* and *M. tengra* fish. No significant different between *C. striatus* and *C. punctatus* in case of ash content whereas in case of FFA value, *C. striatus* is insignificant with both *C. punctatus* and *M. tengra* but between *C. punctatus* and *M. tengra* they are significantly different. No significant different were present in *C. striatus, C. punctatus* and *M. tengra* in case of pH.

3.2 Mineral composition: Important mineral (mg/100g of Fish) composition of macro and micro elements in three experimental fish in fresh condition is reported in Table-2.

Table 2: Important mineral (mg/100g of Fish) composition of macro and micro elements in experimental fresh fish, *Channa striatus*, *Channa punctatus* and *Mystus tengra*

Experimental Fishes	Macroelements		Micro/Trace elements			
	Ca	Mg	Fe	Cu	Zn	Mn
C. striatus	11.2	10.125	1.475	0.7	0.25	0.1
C. punctatus	16.35	9.425	1.275	0.65	0.425	0.05
M. tengra	22.025	11.4	2.25	0.55	1.275	0.125

The macro elements Ca, Mg were abundant in all the fishes examined while micro elements Cu, Fe, Zn, Mn were present in trace amounts. Their abundant presence may be due to the facts that the body needs these macro elements in more amounts than the micro elements in the structure and function of the body and that the concentrations in the water body is very low.

Fresh *Channa striatus*, *Channa punctatus* and *Mystus tengra* fish had calcium (Ca) and magnesium (Mg) content of 11.2, 16.35 & 22.025mg/100g and 10.125, 9.425, and 11.4 mg/100g respectively. Small fishes are mostly eaten with bones, so the amount of available calcium is high in *Mystus tengra*fish than in other two fishes. Ca is essential in human body for the formation of bones,teeth, muscle tone and nervous impulse [29, 30]. It has been reported that, fractional Ca absorption in human body is 24% [31].Comparatively higher concentration of Mg was observed in *Mystus tengra* fish than other fish, which could be linked to the Mg deposit in the fish bone. In that case, *Mystus tengra*fish as a whole one may be considered as one of the good source of Ca and Mg in the diet.Normal extra cellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances [32].

The trace element contents in the fishes examined recorded very low in trace amounts. Fe content among the trace elements was dominant in these three fishes. Fe content was highest (2.25 mg/100g) in *Mystus tengra*and lowest (1.275 mg/100g) in *Channa punctatus*. (Fe) Iron has the longest and best history among all the micronutrients. It is key elements in the metabolism of almost all living organisms.Iron is necessary component for the formation of hemoglobin, which is required to form red blood cells [33].Fresh *Channa striatus*, *Channa punctatus* and *Mystus tengra* fish had Zn content of 0.25, 0.425 & 1.275; Cu content of 0.7, 0.65 & 0.55; Mn content of 0.1, 0.05 & 0.125 mg/100g respectively. The presence of zinc in the fishes could mean that the fishes can play valuable roles in the management of diabetes, which result from insulin malfunction [32].

From this study mineral elements detected were in the order Ca>Mg>Fe>Cu>Zn>Mn for *C. striatus* and *C. punctatus* whereas Ca>Mg>Fe>Zn>Cu>Mn for *M. tengra*.Similar order of magnitude of the trace elements was reported in fillets of several species of fresh water fishes [34]. Fawole reported decreasing order of Zn>Fe>Ni>Cu>As in the studies of some fresh water species [35]. Gopakumar reported the values of major elements of Indian fishes in the above same order in most fishes [36].

The mineral contents of each species is a function of the availability of these elements in their local environment, diet absorptive capability and as well as their preferential accumulation. It is therefore become important to equally consider the minerals status of fish and the persistence food safety of the fish prior to consumption in addition to the prevailing choice of taste, size, type and external morphology of fish.

3.3 Bacteriological study: The total bacterial count (SPC) was found to be 2×10^5, 1.1×10^5 and5×10^5 CFU/g in *C. striatus, C. punctatus* and *M. tengra* fish which is presented in Table- 3.

Table 3: Total Bacterial count (CFU/g) in experimental fresh fish, *Channa striatus*, *Channa punctatus* and *Mystus tengra*

***Channa striatus*(CFU/g)**	***Channa punctatus*(CFU/g)**	***Mystus tengra*(CFU/g)**
2×10^5	1.1×10^5	5×10^5

Although it is widely accepted that the initial microbial load of freshwater fish varies depending on water conditions and temperature, most of the available literature on different freshwater and marine water species (Tilapia, Striped bass, Rainbow trout, Silver perch and Sea bream) reports bacterial counts of 2 to 7 Log CFU/g [37]. The acceptable limit for bacterial count is 5×10^5/g for fresh fish [38]. The bacterial count in this experiment with three fish species showed the values within the range of acceptable limit.

4. Conclusions

Fish is a highly proteinous food consumed by the populace. A larger percentage of consumers eat fish because of its availability, flavors, palatability while fewer percentage do so because of it's nutritional value. This present work has elucidated more on the importance of freshwater fishes as good sources of protein and minerals. It has also broadened our knowledge on the nutritional value of some freshwater fish speciesand in this point of view, it can be suggested that taste, size, freshness and other related external appearances should not be the only factors to be considered in making choice for marketing and consumption of fishes.However, it was discovered that, some microbes present but their count was in low range. Also Regarding the suitable amount of lipids and

nutrient contents, it appears that *C. striatus*, *C.punctatus* and *M. tengra* of river Meghna could be used as human diet.

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